NaCl; pH 6.0 at 60 cm/hr. The collected protein in the elution pool was stored at 2-8° C. until the next purification step was carried out.

Purity levels achieved, as determined by SEC and RP-HPLC are shown in FIG. **5**.

Following the separation, the resin media was cleaned in-place by flowing 3 CV of 1 M sodium hydroxide, at 120 cm/hr and held for 60 minutes prior an additional 3CV wash with 1 m sodium hydroxide.

The results of this separation demonstrate that an insoluble protein expressed in a non-mammalian system can be captured and purified from a refold buffer with a variety of separation matrices, including an ion-exchange separation matrix.

Example 4

Re-Usability of Protein A Affinity Resin Used to Isolate a Fc-Containing Protein Directly from a Refold Buffer by Affinity Chromatography

In another aspect of the method, a range of column cleaning methods can be employed in conjunction with the methods described herein, allowing the chromatography resins to be reused to an extent that make the method economically feasible. As described in Examples 2 and 3 for the case of Protein A affinity resins, cleaning protocols have been developed and demonstrated to remove product and non-product contaminants from the resin to allow reuse. The cleaning agents include caustic (e.g. sodium or potassium hydroxide), detergents (e.g. SDS or Triton X-100), denaturants (e.g. urea or guanidine-derivatives), and reductants (e.g. DTT, or thioglycolates). These agents can be used in combination or alone.

In order to demonstrate the reusability of column resins following application of the direct capture methods described, an aliquot of pH adjusted and filtered Fc-containing protein was loaded on new, unused resin and resin that had been previously cycled 94 times to evaluate the cleaning of the Protein A resin and the effect on purification binding and separation of an Fc-containing protein with regard to resin history.

The media was equilibrated with 5 column volumes (CV) of 25 mM Tris, 100 mM sodium chloride; pH 7.4, or similar buffered solution. An aliquot of protein sampled directly from a refold buffer was filtered through a series of depth and/or membrane filter to remove particulates. The conditioned and filtered protein mixture was then loaded on the column to approximately 0.35 millimoles total protein/mL resin at a 6-10 minute residence time. See FIG. **1**, which correlates protein bound and protein loaded as a function of residence time.

After loading, the column was washed with 25 mM Tris, 100 mM sodium chloride; pH 7.4, or similar buffered solution, for 4.5 CV at up to 400 cm/hr. The protein of interest was recovered from the resin by eluting with 100 mM sodium acetate, pH 3.7 at up to 300 cm/hr. Each column was regenerated using 5CV phosphoric acid and 5 CV of an acidic buffered solution containing 50 mM Tris, 10 mM citrate, 6 M urea, and 50 mM DTT; pH 7.4.

This procedure was repeated for greater than 100 cycles. Selected samples from this reuse study were submitted for SEC-HPLC analysis. The goal was to track the % MP purity, % HMW and % dimer species from the pools as well as to understand the change of purity level from the load. No major differences were observed between the used columns and new columns.

This Example demonstrates that not only can a complex protein be captured from a complex chemical solution, but that the resin can be cycled repeatedly and cleaned and reused reproducibly over a number of industrially-relevant cycles.

What is claimed is:

1. A method of purifying a protein expressed in a non-native soluble form in a non-mammalian expression system comprising:

(a) expressing a protein in a non-native soluble form in *E. coli;*

(b) lysing the *E. coli* to generate a cell lysate;

(c) contacting the cell lysate with a separation matrix under conditions suitable for the protein to associate with the separation matrix;

(d) washing the separation matrix; and

(e) eluting the protein from the separation matrix.

2. The method of claim **1**, wherein the protein is a complex protein.

3. The method of claim **2**, wherein the complex protein is selected from the group consisting of: a multimeric protein, an antibody, and an Fc fusion protein.

4. The method of claim **1**, wherein the separation matrix is an affinity resin selected from the group consisting of: Protein A, Protein G, and a synthetic mimetic affinity resin.

5. The method of claim **1**, wherein the separation matrix is a non-affinity resin selected from the group consisting of: ion exchange, mixed mode, and a hydrophobic interaction resin.

6. The method of claim **1**, wherein the cell lysate is filtered before it is contacted with the separation matrix.

7. The method of claim **1**, further comprising:

(f) refolding the protein to its native form.

8. A method of purifying a protein expressed in a non-native limited solubility form in a non-mammalian expression system comprising:

(a) expressing a protein in a non-native limited solubility form in a non-mammalian cell;

(b) lysing the non-mammalian cell;

(c) solubilizing the expressed protein in a solubilization solution comprising one or more of the following:

(i) a denaturant;

(ii) a reductant; and

(iii) a surfactant;

(d) forming a refold solution comprising the solubilized protein and a refold buffer, the refold buffer comprising one or more of the following:

(i) a denaturant;

(ii) an aggregation suppressor;

(iii) a protein stabilizer; and

(iv) a redox component;

(e) applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the separation matrix;

(f) washing the separation matrix; and

(g) eluting the protein from the separation matrix to yield a purified protein.

9. A method of purifying a protein expressed in a non-native soluble form in non-mammalian expression system comprising:

(a) expressing a protein in a non-native soluble form in a non-mammalian cell;

(b) lysing the non-mammalian cell to generate a cell lysate;